Video Article

Testing *Drosophila* Olfaction with a Y-maze Assay

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Abstract

Detecting signals from the environment is essential for animals to ensure their survival. To this aim, they use environmental cues such as vision, mechanoreception, hearing, and chemoperception through taste, via direct contact or through olfaction, which represents the response to a volatile molecule acting at longer range. Volatile chemical molecules are very important signals for most animals in the detection of danger, a source of food, or to communicate between individuals. *Drosophila melanogaster* is one of the most common biological models for scientists to explore the cellular and molecular basis of olfaction. In order to highlight olfactory abilities of this small insect, we describe a modified choice protocol based on the Y-maze test classically used with mice. Data obtained with Y-mazes give valuable information to better understand how animals deal with their perpetually changing environment. We introduce a step-by-step protocol to study the impact of odorants on fly exploratory response using this Y-maze assay.

Video Link

The video component of this article can be found at http://www.jove.com/video/51241/

Introduction

Chemoreception through taste or olfaction is a key sensory modality for animal survival. It gives vital cues necessary for the detection of a danger or food sources, as well as for social interactions. It also helps animals to find a sex partner necessary for their reproduction. For more than 20 years, intensive research, including Nobel prize winning work by Richard Axel and Linda Buck in 2004 "for their discoveries of odorant receptors and the organization of the olfactory system", has been carried out to reveal the molecular and cellular bases of olfaction[1,2].

One of the favorite animal models for scientists to dissect olfactory perception is *D. melanogaster*. This insect shares a similar cellular and molecular odor-coding strategy with mammals. The scientific community uses diverse behavioral paradigms to study the role of odorants in this fruit fly. These tests include multimodal assays such as courtship tests where various sensory modalities, including olfaction, are important to elicit male courtship[3]. Other assays have also been developed to tackle the role of odorants more specifically; these include T-mazes, Y-mazes, trap assays, four-field arenas and wind-tunnels[4,5,6,7,8].

In this article we present a simple modified Y-maze assay, which provides robust olfactory responses using *D. melanogaster*. Our set-up uses end-tips in contrary to a previously described method[9]. Thus, our Y-maze has two advantages. First, it avoids any return in the system once the fly has made her choice. Second, it limits the exchange of odorants in all areas of the Y-maze. This last advantage is important since *Drosophila* are very sensitive to air flow which is often used to avoid odorant saturation. To adjust the experimental set-up with an air flow would be time and cost consuming. Therefore, our Y-maze assay represents an efficient and fast way to test olfactory performance of *Drosophila*.

Protocol

1. Before Starting

1. Use an isogenized reference stock bearing stable and robust behavioral phenotypes. There is no general rule for choosing this stock, since all potential controls may carry heterogeneous background alleles.
2. Use this control strain to backcross every other stock necessary for later steps. This backcrossing step is typically represented by at least 5 successive crosses of a single virgin female (to allow possible crossing-over between homologous chromosomes) to 2–3 isogenic reference males[5]. This step is important to homogenize the genetic background between the different fly stocks.
3. Maintain *Drosophila* stocks on a standard corn flour (9%), yeast (10%), and agar medium (1.5%) complemented with antibiotic (0.4% methyl para-hydroxy-benzoate) in a 12-hr light/dark cycle at 25 °C.

4. Achieve chemosensory experiments in a temperature-controlled room (25 °C) under far red light (to eliminate the contribution of visual cues, and to focus on chemosensory signals). Regularly renew the air of the room to ventilate the area between each experiment.

2. Olfactory Response using a Y-maze Assay

1. Starve the flies for 16–18 hr at 25 °C in glass tubes containing wet paper towel before testing.
2. Join a Y-shape connector to two glass vials and to a smaller plastic vial (loading vial). Use 1 ml pipette tips that pass through the foam stoppers to link the connector to the three vials, and to obtain a tightly sealed Y-maze. Cut the narrow ends of two pipette tips (~2 mm diameter, to avoid any return of the fly once it has made its decision) to form two “trap” vials, and a large end of one pipette tip to form the “loading” tube (**Figure 1A**).
3. Just before connecting the “trap” vials (**Figure 1B**), place one ~6 mm diameter filter paper in each vial. Add 40 µl of odorant solution on one filter paper, and 40 µl of the corresponding solvent on the second filter paper.
4. Introduce ten 4 to 9 day-old flies into the “loading” vial. Do not use CO_2 anesthesia during this transfer, since it has a strong effect on behavior[10]. Rather use brief cooling on ice. Proper manipulation of anaesthetized flies is important to limit stress on the subjects as much as possible.
    1. Perform a series of Y-maze tests at 25 °C under far-red light (using LED bulbs to limit possible heating source) to avoid visual stimuli as much as possible. Be careful to alternate the orientations of the Y-mazes (odorant containing tube on the left, or on the right, and loading tube in front or in the back; **Figure 1C**).
    2. Allow several hours for the flies to enter in the trap vial containing the odorant or the solvent. Count flies after 24 hr to increase the participation up to more than 80% and provide the maximum olfactory index value (Simonnet, personal communication).
    3. Calculate the resulting olfactory index using the following formula: (number in the odor tube - number in the solvent tube)/total number of loaded flies.
5. Wash Y-maze set-up as follows: soak the dismantled set-up in RBS 35 MD overnight. Thoroughly rinse out with tap water. Finally rinse with deionized water and let dry out.

3. Statistical Analysis of the Data

1. Perform a t-test, a one-way ANOVA or a two-way ANOVA depending on data and variables.

Representative Results

Figure 1D shows two representative responses using this Y-maze assay. Canton-S males strongly avoid 10% acetic acid diluted in distilled water, whereas they do not significantly avoid 10% phenylacetic acid. These assays are based on 10 males per replicate placed together in the loading vials. This protocol can sometimes lead to large standard error of the mean. If needed, it is possible to reduce this drawback by using 20 males per replicates instead of only 10. Mathematically, the choice of one individual has a lower weight on the index value for a larger sample size.

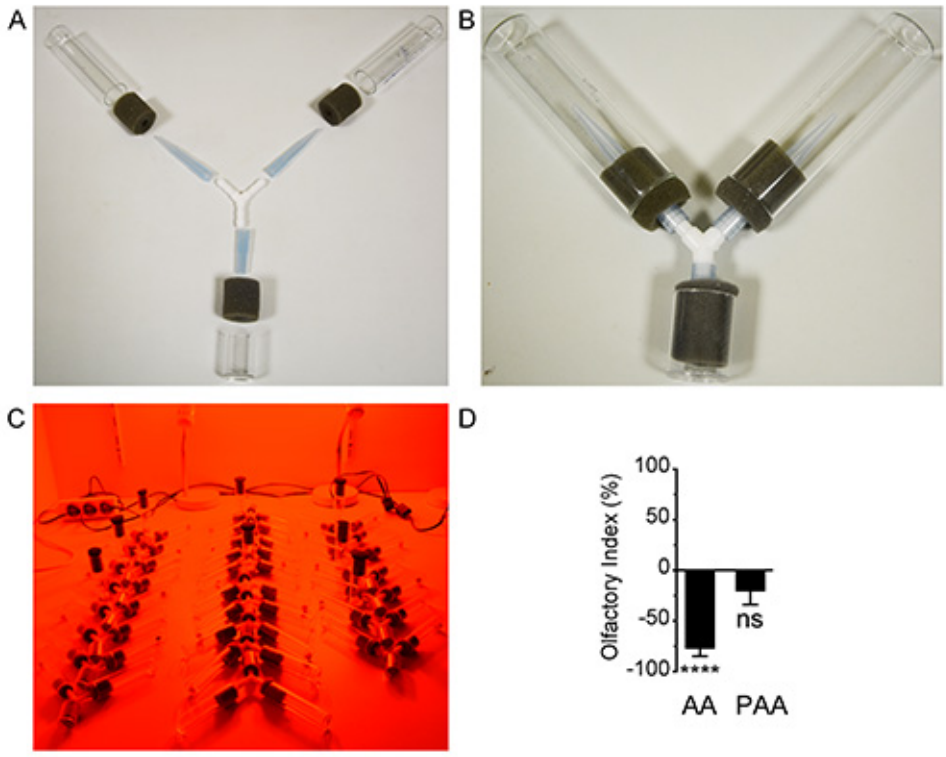

Figure 1. Male olfactory response assessed with a Y-maze set-up. A) Split device. **B)** Assembled device. **C)** Set-up in working condition under far-red light. **D)** Quantification of male olfactory responses toward acetic acid (AA) or phenylacetic acid (PAA) both diluted in distilled water (10% v/v) (N = 11, representing a total of 110 flies). Statistical analysis was performed using a t-test comparing the data to 0. 0 means no preference. A negative value indicates an aversion to the odorant, and a positive value an attraction. ****: $p < 0.0001$; ns: non-significant ($p = 0.1680$). Please click here to view a larger version of this figure.

Discussion

Our Y-maze protocol is based on a previously described protocol[9]. However, we introduce two major differences. First, we use narrow pipette tips to prevent the flies from returning once they decide to enter in the vial containing the solvent or the solvent plus the odorant. These narrow tips are also useful to limit the odorant diffusion in the Y-maze. Second, we use a smaller loading vial to force the flies to enter in the Y-maze. It is important to have a high participation of these flies (80% to 100% after 24 hr; Simonnet, personal communication).

This Y-maze assay represents an efficient test to evaluate chemosensory responses in *Drosophila*. The context of the test, including stress on the flies (from air flow, manipulation of the flies during the loading step, etc.) is potentially influencing their olfactory responses. These environmental issues are critical and could explain, at least partially, why different studies could have different behavioral outcomes. For example acetic acid is shown to be repulsive in some conditions [4,11], whereas it is attractive in others[12]. Therefore it is critical to control all of these parameters as much as possible.

One possible limitation of this Y-maze assay is its artificial context for flies since they are held in a locked design. Additionally, flies have to enter in narrow passages, which might be stressful for them. The experimenter has to remember this when interpreting the data.

To bypass these possible limitations, other complementary olfactory tests could be performed to confirm the impact of odorants on behavior. These tests include T-mazes[4], 4 field-arena assays[7], or wind tunnels[8]. However, these tests also utilize artificial environments for flies, which could be more or less stressful. For example, during T-maze tests flies are highly stressed since they are shaken during the loading step[4]. However, an advantage of a T-maze compared to this Y-maze assay is that flies have to choose within minutes where to go (to the odorant or to the solvent). Therefore the Y-maze response represents a “reflective” choice, whereas outcomes obtained with a T-maze represent a reflex choice in a highly stressful condition.

Finally a possible improvement of this Y-maze assay would be to use glass tubes all along the set up instead of plastic for some components (loading tube, connector, pipette tips). These plastic parts can, in theory, have a smell since they are made from petrol.

Disclosures

The authors have nothing to disclose.

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